Production of Motif-Specific Context-Independent
Antibodies Using Peptide Libraries as Antigens
Comb, et al
Atty. Docket No: CST-138 CIP2
Sheet 1 of 25

FIG. 1/

				ANTIBODY DILLITIONS	25			100
PEPTIDE	SECUENCE	1.00E+03	5.00E+03	1.00E+04	5.00E+04	1.00E+05	5.00E+05	1.005
					1	1	3	6
Ŧ.	x.x.x.x.mr-x.x.x.x.c/s	1.92	1.32	0.54	0.34	0.0	5.0	0.02
							1	
Ser-Thr	XXXXXXXSerThrXXXXXXOys	0.11	0.05	0.01	0.0	0.01	0.00	0.00
							1	100
Threonine* mix	18 phospho-Thr peptide	1.84	1.13	0.40	0.26	0.10	0.07	0.03
								1
Serine* mix	38 phospho-Ser peptide	0.12	0.04	0.05	0.02	0.05	0.01	0.00
							1	
AM-Thraos-P	IIe-Lya-Asp-Giy-Ala-Thr-Met-Lya-Thr-Phe-Cya-Giy-Thr-Pro	1.18	0.65	0.24	0.13	0.03	0.01	0.00
	(SEO ID NO:1)							
APP1-Thr668-P	Asp-Ala-Ala-Val-Thr'-Pro-Lys-Ayg-His-Leu-Ser-Lya-Cys	0.14	0.03	0.01	0.01	0.01	9.0	0.00
	(SEO ID NO:2)							
38	Ass-Thr-Gin-le-Lvs-Arc-Asn-Thr-Phe-Val-Giy-Thr-Pro-Phe-Cys	1.71	1.13	0.39	0.22	0.03	0.02	0.02
3	(SEC ID NO:3)							
	112 OL MAI TANA TON TON TON TON	1.77	1.15	0.41	0.27	0.06	0.03	0.0
CAK-Innib/-r								
	(SEQ ID NO. 4)	1	96 1	0.83	0 40	0.09	0.05	0.01
CAMIV-Thr188-P	His-Gin-Val-Leu-Met-Lys-Thr*-Val-Cys-Gly	2	95.	3				
	(SEQ ID NO:5)							6
CDC2-Thr167-P	IIe-Pro-IIe-Arg-Val-Tyr-Thr"+Hia-Giu-Val-Val-Thr-Leu-Cys	1.02	0.56	0.14	0.08	0.03	0.0	0.0
	(SEQ ID NO:6)							18
CDK2.Thr159.P	GN-Val-Pro-Val-Arg-Thr-Thr-His-Glu-Val-Val-Thr-Leu-Cys	1.88	1.79	0.51	0.44	0.08	0.04	0.02
	(SEQ ID NO:7)							
-70SBK-Thr389-P	Agn-Gin-Val-Phe-Leu-Giv-Phe-Thr-Tyr-Val-Ala-Pro-Lys-Lys-Cys	1.99	1.44	0.62	0.39	0.08	0.04	0.01
	(SEQ ID NO:8)							
PKCalpha-P	Lys-Glu-His-Met-Met-Asp-Gly-Val-Thr-Thr-Arg-Thr"-Phe-Cys	1.82	1.63	0.94	0.58	0.15	0.08	0.05
	(SEQ ID NO:9)							
g Was	Aso-His-Thr-Giv-Phe-Leu-Thr*-Glu-Tyr*-Val-Ala-Thr-Arg-Trp-Cys	1.56	1.18	0.51	0.30	0.07	0.04	0.02
	(SEO ID NO:10)							
Min SarSA/62.P	Glid and an Pro-ThrPro-Pro-Lau-Ser-Pro-Ser-Arg-Arg-Arg-Arg-Arg-Arg-Arg-Arg-Arg-Ar	0.11	0.05	0.03	0.02	0.02	0.05	0.05
	(SEQ ID NO:11)							
P38.2P	Leu-Ala-Arg-His-Thr-Asp-Asp-Glu-Met-Thr*-Gly-Tyr*-Val-Ala-Thr-Arg-Cys	0.54	0.30	0.08	0.08	0.04	0.04	0.02
i	(SEQ ID NO:12)							000
JNK-2P	Ser-Phe-Met-Met-ThrPro-TyrVal-Val-Thr-Arg-Tyr-Tyr-Arg-Cys	1.49	0.44	0.12	0.07	0.03	0.05	0.02
in the state of th	(SEO TH NO:13)							

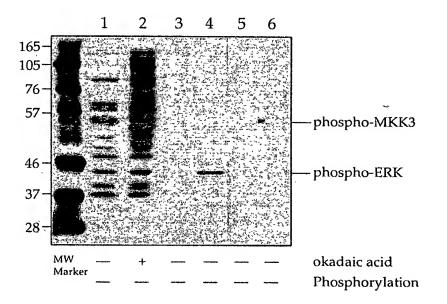
(SEQ ID NO:13)

FIG. 1B

PEPTIDE SEQUENCE		phospho-Thr Reactivity
XXXXXXS*XXXXXXX		
XXXXY*XXXX		_
XXXXXPXS*/T*PXR/KXXX	(SEQ ID NO:14)	+ +
XXXXRSXS*XPXXXX	(SEQ ID NO:15)	_
XXXXRSXSXPXXXX	(SEQ ID NO:16)	_
XXXXXPXS*/T*PXXXXX	(SEQ ID NO:17)	+ +
XXXXXPXS/TPXXXXXX	(SEQ ID NO:18)	
XXXXXT*XXXXXXX		+ + +
XXXXXXS/TXXXXXXX		
21 phospho-Thr peptides mixtur	е	+++
38 phospho-Ser peptides mixtur	е	_
30 phospho-Tyr peptides mixtur	е	<u> </u>
NEB LIBRAR		
X-X-X-X-D/E-X-X-S*-T*-X-X-X	K-X-X-C(SEQ ID NO:	+++
X-X-X-X-X-X-S*/T*-D/E-D/E-	-D/E-X-X-X (SEQ ID	NO:20) + +
X-X-X-X-F-X-X-F-S*/T*-F/Y->		+++
X-X-X-X-R/K-X-S*/T*-X-X-X-X-	X-X-X-C (SEQ ID	NO:22) + + +
X-X-X-R/K-X-X-S*/T*X-X-X-X		• •
X-X-X-X-X-X-S*/T*-F/I/M-X->		
X-X-X-X-X-X-S*/T*-F/I-X-X-X	-X-X-C (SEQ ID	NO:25) + + +
X-X-X-X-X-X-S*/T-P-X-X-X-X	-X-X-C (SEQ ID	NO:26) + +
X-X-X-X-X-T*-X-X-X-X-X-X-C		+++
X-X-X-X-P-X-S*/T*-P-X-X-	X-X-X-C (SEQ ID NO:	27) + +
X-X-X-X-X-X-S/T-X-X-X-X-X-X-X-X-X-X-X-X-X-X-X-X-X-X-X	X-C (SEQ ID NO:	28) —
X-X-X-X-X-P-X-S*/T*-P-X-R/	K-X-X-C (SEQ ID	NO:29) + +
Al	ITIBODY REACTIVITY	ELISA O.D.
+	+ + very strong	> 2
	++ strong	1-2
	+ weak - very little	0.2 - 1 < 0.2

Production of Motif-Specific Context-Independent Antibodies Using Peptide Libraries as Antigens Comb, et al Atty Docket No. CST-138 CIP2 Sheet 3 of 25

FIG. 1C



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FTG. 10

			-5-	-5-4-3-2-1		+1+2+3+4+5	.5	
Fixed			×	SXXXX	er*/Th	XXXXXSer*/Thr*XXXXX	×	
Amino			Fixed AA po	osition relati	ve to ph	Fixed AA position relative to phospho-Ser*/Thr*	Thr*	
Acid	- 4	- 3	-2	1 -	S*/T*	+ 1	+2	+ 3
Ala	+	++	+ + +	+++		+++	+++	++
Cys	+++	+ + +	+++	+++		++++	+++	+
Asp	++	++	++	+++		++++	+++	++
Glu	++	++	++	+++		++++	+++	++
Phe	+ +	+ +	++	+++		+++	++++	++
Gly	++	++	++	++		+++	+++	+
His	+ +	++	++	+++		+++	+++	++
lle	+ +	+ +	++	++++		+++	+++	++
Lys	+ +	+ +	+ +	+++		+++	++++	+++
Leu	+	+ +	+ +	+++		+++	+ + +	++
Met	++	+ +	++	+++		+ + +	+++	++
Asn	++	+++	++	+++		+++	+++	+
Pro	++	+ +	+ +	+++		-	+++	++
Gh	++	+ +	++	+ + +		+++	+++	+
Arg	+	+	++	+++		+++	+++	++
Ser	+	++	+ +	+		+ + +	++++	+
Thr	+	++	+ +	+++		+++	+++	++
Val	++	++	+ +	+++		+++	+++	++
Trp	++	+ +	+ +	+++		+ + +	+++	+
Tvr	++	+	+	+++++++++++++++++++++++++++++++++++++++		1	7 7 7	1

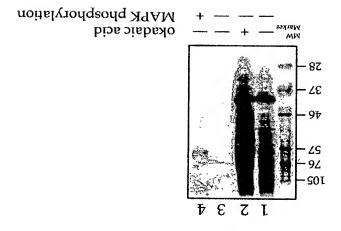
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FTG 2A

				ANTIBODY DILUTIONS	ILUTIONS			
PEPTIDE	SEQUENCE	1.00E+03	5.00E+03	1.00E+04	5.00E+04	1.00E+05	5.00E+05	1.00E+06
РХЗРР	X-X-X-X-Pro-X-Ser*-Thr*-Pro-X-X-X-X-X-Cys	1.82	1.97	1.74	1.40	0.70	0.35	80.0
	(SEQ ID NO:27)							00.0
Threonine mix	18 phospho-Thr peptide mix	1.97	1.37	0.67	0.36	0.13	0.07	0.05
Ser/Thr	X-X-X-X-X-X-Ser/Tu-X-X-X-X-X-Cys	0.14	0.03	0.01	0.00	00.0	00 0	000
	(SEQ ID NO:28)							3
RB Thr373-P	Val-IIe-Pro-Pro-His-Thr*-Pro-Val-Ag-Thr-Val-Met-Asn-Thr-Cvs	2.07	2.17	1 70	1 20	0 48	0 18	0 0
	(SEQ ID NO:30)					2	2	3
MKK3-Thr-P	Ser-Val-Ala-Lys-Thr*-Met-Asp-Ala-Gly-Cys	0.08	0.04	0.01	6	6	00 0	000
	(SEQ ID NO: 31)						3	8
PKCalpha-P	Lys-Glu-His-Met-Met-Asp-Gly-Val-Thr-Thr-Arg-Thr"-Phe-Cys	0.05	0.02	0 0	0 0	0	000	000
	(SEQ ID NO:9)						2	
p70 S6K-Thr388	p70 S6K-Thr389 Asn-Gin-Val-Phe-Leu-Giy-Phe-Thr*-Tyr-Val-Ala-Pro-Lys-Lys-Cys	0.11	0.05	0 0	00 0	0 0	000	000
	(SEQ ID NO:8)			5	8	2	9	9
cdk4-Thr172-P	cdk4-Thr172-P Arg-lie-Tyr-Ser-Tyr-Gin-Met-Ala-Leu-Thr*-Pro-Val-Val-Lys-Cys	2.07	2.21	2.01	1.55	0.69	0.31	0.07
	(SEQ ID NO:32)							

Production of Moitf-Specific Context-Independent Antibodies Using Peptide Libraries as Antigens Comb, et al Atty. Docket No: CST-138 CIP2 Sheet 6 of 25

FIG. 28

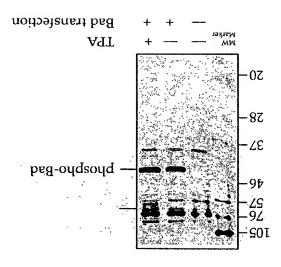


Production of Motif-Specific Context-Independent Antibodies Using Peptide Libraries as Antigens Comb, et al Atty. Docket No: CST-138 CIP2 Sheet 7 of 25

FTG 3A

				ANTIBODY DILUTIONS	LUTIONS		
DEDMINE	SORIOS	1,00E+03	5.00E+03	1.00E+04	5.00E+04	1.00E+05	5.00E+05
14.3.3 BM-P	X-X-X-Arg-Ser-X-Ser-X-Pro-X-X-X-Cys	2.41	2.15	1.49	1.15	0.44	0.25
	(SEQ ID NO:33)						
14-3-3 BM	X.X.X.X.Arg-Ser-X-Ser-X-Pro-X.X.X.Cys	0.07	0.03	0.05	0.03	0.05	0.03
	(SEQ ID NO:34)						
CDC25-Ser216-P	10	2.35	2.08	1.49	1.05	0.33	0.18
CDC25-Ser216	GIV-Leu-Tyr-Arg-Ser-Pro-Ser-Met-Pro-Glu-Asn-Leu-Asn-Arg-Cys	0.02	0.05	0.03	0.03	0.04	0.03
	(SEQ ID NO:36)						
Bad-Ser112-P	Thr-Arg-Ser-Arg-His-Ser-Ser'-Tyr-Pro-Ala-Gly-Thr-Glu-Glu-Cys	1.59	0.43	0.10	0.03	0.01	0.00
	(SEQ ID NO:37)						
Bad-Ser112	Thr-Arg-Ser-Arg-His-Ser-Ser-Tyr-Pro-Ala-Giy-Thr-Glu-Glu-Cys	0.00	0.00	0.00	0.00	0.00	0.00
	(SEQ ID NO:38)						
Bad-Ser136	Phe-Arg-Gly-Arg-Ser-Arg-Ser-Ala-Pro-Pro-Asn-Leu-Trp-Ala-Cys	0.03	0.00	00'0	0.00	0.00	0.00
	(SEQ ID NO:39)						
Bad-Ser138-P	Phe-Arg-Gly-Arg-Ser-Arg-Ser*-Ala-Pro-Pro-Asn-Leu-Trp-Ala-Cys	3.25	1.86	0.73	0.51	0.07	0.03
	(SEQ ID NO:40)						

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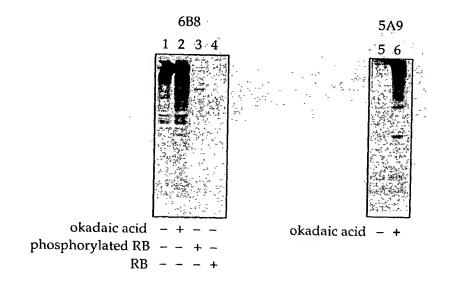
Production of Motif-Specific Context-Independent Antibodies Using Peptide Libraries as Antigens Comb, et al Atty. Docket No: CST-138 CIP2 Sheet 9 of 25

FIG. 4A

SEQUENCE 6B8 6A9 SearThirPro-P XXXXXXXXSerThir-Pro-XXXXXXXQs 1.774 0 ProXSerThirPro-P (SEQ ID NO:26) 0.924 0 ProXSerThirPro-P XXXXXXXPro-XSerThir-Pro-XXXXXXXQs 0.02 0 ProXSerThirPro-P (SEQ ID NO:27) 0.02 0 ProXSerThirPro-P XXXXXXXPro-XSerThir-Pro-XXXXXXXQs 0.02 0 Thir-P XXXXXXXThir-Pro-XXXXXXXQs 0.031 0 Sar-P XXXXXXXXXXXXXXQs 0.021 0 Tyr-P XXXXXXXXXXXXXXXXQs 0.023 0 Tyr-P XXXXXXXXXXXXXXXXXXQs 0.023 0 Rb (Sar795)-P Sar-Po-Tyr-Lys-Pha-Pro-Ser-Ser'-Pro-Leu-Arg-Ile-Pro-Gly-Cys 0.023 0 Rb (Thir37)-P (SEQ ID NO:30) 0.023 0 Rb (Thir37)-P (SEQ ID NO:30) 0.022 0 Rb (Thir37)-P (SEQ ID NO:30) 0.022 0 Rb (Thir37)-P (SEQ ID NO:30) 0.022 0 Rb (Thir373) Val-lie-Pro-Pro-His-Thir-Val-Mat-Asn-Thir-Cys 0.022 <th></th> <th></th> <th>MONOCLONAL ANTIBODIES</th> <th>ANTIBODIES</th>			MONOCLONAL ANTIBODIES	ANTIBODIES
X.X.X.X.Sar'Thr-Pro-X.X.X.X.Cys	БЕРПІЛЕ	SEQUENCE	688	5A9
X.X.X.X.X.Ser/Thr-Pro-X.X.X.X.Cys				
(SEQ ID NO:26) 0.924 ro-P X.X.X.X.Pro.X.Ser/ThrPro.X.X.X.Cys 0.02 ro-P X.X.X.X.Pro.X.Ser/ThrPro.X.X.X.X.Cys 0.02 ro-P X.X.X.X.X.Pro.X.Ser/ThrPro.X.Arg/Lys-X.X.Cys 1.955 roxArg-P X.X.X.X.X.Pro.X.Ser/ThrPro.X.Arg/Lys-X.X.Cys 0.031 roxArg-P X.X.X.X.X.X.X.X.X.Cys 0.021 x.X.X.X.X.X.X.X.X.X.X.Cys 0.023 x.X.X.X.X.X.X.X.X.X.Cys 0.023 x.X.X.X.X.X.X.X.X.X.X.X.Cys 0.023 x.X.X.X.X.X.X.X.X.X.X.X.Cys 0.023 x.X.X.X.X.X.X.X.X.X.X.X.X.X.X.X.X.X.X.X	Ser/ThrPro-P	X-X-X-X-X-Ser/Thr-Pro-X-X-X-X-X-Cys	1.774	0.731
SEQ ID NO:27 SEQ ID NO:27 SEQ ID NO:27 SEQ ID NO:27 SEQ ID NO:41 SEQ ID NO:41 SEQ ID NO:41 SEQ ID NO:42 SEQ ID NO:43 SEQ ID NO:44 SEQ ID NO:44		(SEQ ID NO:26)		
(SEQ ID NO:27) 0.02	ProXSer/ThrPro-P	X-X-X-X-X-Pro-X-Set/Thr*-Pro-X-X-X-X-X-Cys	0.924	0.766
SEQ ID NO:41)		(SEQ ID NO:27)		
(SEQ ID NO:41)	ProXSer/ThrPro-P	X-X-X-X-X-Pro-X-Ser/Thr-Pro-X-X-X-X-Cys	0.05	0.063
SEQ ID NO:42 1.955		(SEQ ID NO:41)		
(SEQ_ID_N0:42)	ProXSer/ThrProXArg-P	X-X-X-X-X-Pro-X-Ser*/Thr*-Pro-X-Arg/Lys-X-X-X-Cys	1.955	1.275
X-X-X-X-X-Thr*-X-X-X-Cys 0.031		(SEQ ID NO:42)		
X.X.X.X.X.X.X.X.X.X.X.X.X.X.X.X.X.X.X.	Thr-P	X-X-X-X-X-Thr-X-X-X-X-Cys	0	:
nr x-x-x-x-x-x-x-x-x-x-x-x-x-x-x-x-x-x-x-				
nr X.X.X.X.X.X.X.X.X.X.X.X.X.X.X.X.X.X.X.	Ser-P	X-X-X-X-X-X-X-X-X-X-X-X-X-X-X-X-X-X-X-	0.031	0.088
nr X-X-X-X-X-X-X-X-X-X-X-X-X-X-X-X-X-X-X-				
84795)-P Ser-Pro-Tyr-X-X-X-X-X-Cys 0.023 8795)-P Ser-Pro-Tyr-Lys-Phe-Pro-Ser-Ser'-Pro-Leu-Arg-lle-Pro-Gly-Cys 0.032 86795)-P (SEQ ID NO:43) 3.336 86795)-P (SEQ ID NO:43) 3.336 86795)-P (SEQ ID NO:30) 0.02 86795 (SEQ ID NO:44) 0.02	Ser/Thr	X-X-X-X-X-Ser/Thr-X-X-X-X-X-Oys	0.021	0.066
8795)-P Ser-Pro-Tyr-X-X-X-X-X-Cys 0.032 87795)-P Ser-Pro-Tyr-Lys-Phe-Pro-Ser-Ser*-Pro-Leu-Arg-Ile-Pro-Gly-Cys 0.032 8773)-P Val-Ile-Pro-Pro-His-Thr*-Pro-Val-Arg-Thr-Val-Met-Asn-Thr-Cys 3.336 8773) Val-Ile-Pro-Pro-His-Thr*-Pro-Val-Arg-Thr-Val-Met-Asn-Thr-Cys 0.02 68EQ ID N0:44) (SEQ ID N0:44)				
er795)-P Ser-Pro-Tyr-Lys-Phe-Pro-Ser-Ser*-Pro-Leu-Arg-Ile-Pro-Gly-Cys 0.032 hr373)-P Val-Ile-Pro-Pro-His-Thr*-Pro-Val-Arg-Thr*-Val-Met-Asn*-Thr*-Cys 3.336 hr373) Val-Ile-Pro-Pro-His-Thr*-Pro-Val-Arg-Thr*-Val-Met-Asn*-Thr*-Cys 0.02 KSEQ ID NO:44) CSEQ ID NO:44	Tyr-P	X-X-X-X-X-Tyr-X-X-X-X-Cys	0.023	0.072
Ser-Pro-Tyr-Lys-Phe-Pro-Ser-Ser*-Pro-Leu-Arg-lie-Pro-Gly-Cys 0.032 (SEQ ID N0:43) 3.336 Val-lie-Pro-Pro-His-Thr*-Pro-Val-Arg-Thr-Val-Met-Asn-Thr-Cys 3.336 Val-lie-Pro-Pro-His-Thr*-Pro-Val-Arg-Thr-Val-Met-Asn-Thr-Cys 0.02 (SEQ ID N0:44)				
(SEQ ID NO:43) Val-lie-Pro-Pro-His-Thr*-Pro-Val-Arg-Thr*-Val-Met-Asn*-Thr*-Cys (SEQ ID NO:30) Val-lie-Pro-Pro-His-Thr*-Pro-Val-Arg-Thr*-Val-Met-Asn*-Thr*-Cys (SEQ ID NO:44)	Rb (Ser795)-P	Ser-Pro-Tyr-Lys-Phe-Pro-Ser-Ser*-Pro-Leu-Arg-lle-Pro-Gly-Cys	0.032	0.124
Val-Ile-Pro-Pro-His-Thr*-Pro-Val-Arg-Thr-Val-Met-Asn-Thr-Cys 3.336 (SEQ ID N0:30) 0.02 Val-Ile-Pro-Pro-His-Thr-Pro-Val-Arg-Thr-Val-Met-Asn-Thr-Cys 0.02 (SEQ ID N0:44) 0.02		(SEQ ID NO:43)		
(SEQ ID NO:30) Val-lie-Pro-Pro-His-Thr-Pro-Val-Arg-Thr-Val-Met-Asn-Thr-Cys (SEQ ID NO:44)	Rb (Thr373)-P	Val-IIe-Pro-Pro-HIs-Thr*-Pro-Val-Arg-Thr-Val-Met-Asn-Thr-Cys	3.336	3.503
Val-lie-Pro-Pro-His-Thr-Pro-Val-Arg-Thr-Val-Met-Asn-Thr-Cys 0.02 (SEQ ID N0:44)		(SEQ ID NO:30)		
(SEQ ID NO:44)	Rb (Thr373)	Val-IIe-Pro-Pro-His-Thr-Pro-Val-Arg-Thr-Val-Met-Asn-Thr-Cys	0.02	0.073
		(SEQ ID NO:44)		

Production of Motif-Specific Context-Independent Antibodies Using Peptide Libraries as Antigens Comb, et al Atty Docket No: CST-138 CIP2 Sheet 10 of 25

FIG. 4B



Production of Motif-Specific Context-Independent Antibodies Using Peptide Libraries as Antigens Comb, et al Atty. Docket No CST-138 CIP2 Sheet 11 of 25

FIG. 5A

Acetylated BSA(ng) | BSA(ug) | 0.5 | 2 | 10 | 50 | 200 | 500 | 10

FIG. 5B FIG. 5C FIG. 5D

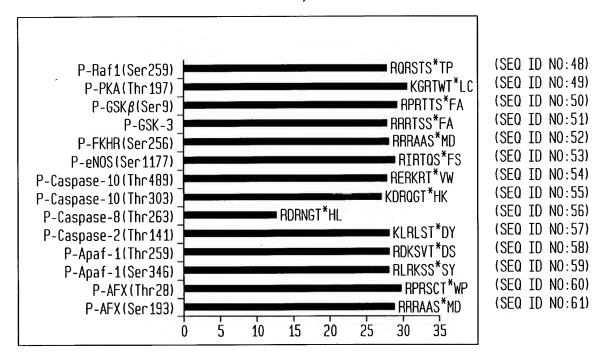
5 6 7 8

9 10 11 12

1 2 3 4

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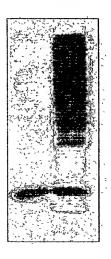
FIG. 6
Signal to noise ratio of ELISA readings using phospho-Akt substrate antibody.



Production of Motif-Specific Context-Independent Antibodies Using Peptide Libraries as Antigens Comb, et al Atty. Docket No CST-138 CIP2 Sheet 13 of 25

FIG. 7

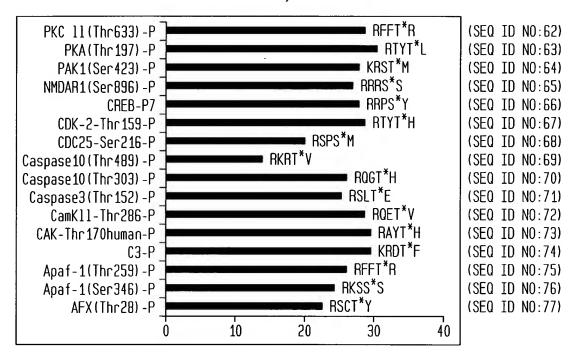
Western analysis of calyculin A-treated A431 cells using Phospho-Akt Substrate Antibody.



- + calyculin A

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FIG. 8
Signal to noise ratio of ELISA reading using phospho-PKA substrates antibody.



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FIG. 9

Western analysis of calyculin A-treated A431 cells using Phospho-PKA Substrate Antibody.

- + calyculin A

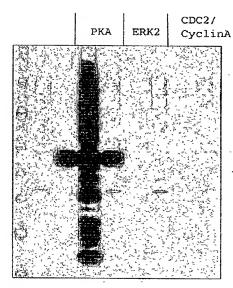


FIG. 10

Western analysis of A431 cell extracts

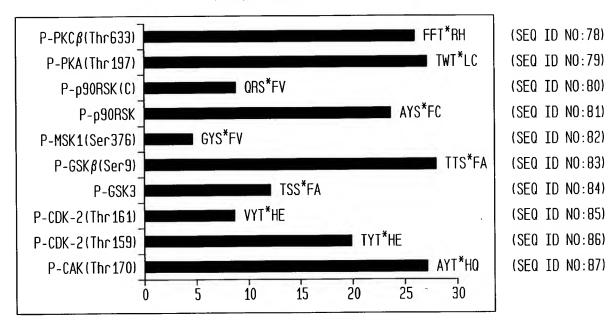
Cell Extracts PKI

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FIG. 11

Signal to noise ratio of ELISA reading using phospho-Serine/threonine phenylalanine antibody.



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FIG. 12

Western analysis of calyculin A-treated A431 cells using phospho-Serine/phenylalanine substrates antibody.



- + calyculin A

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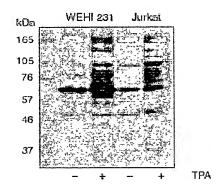
FIG. 13

Signal to noise ratio of ELISA reading using a context-independent phospho-PKC consensus substrate motif antibody.

		0 5 10 15 20 25 30
		والمستخدمة والمستخدم والمستخدمة والمستخدمة والمستخدمة والمستخدمة والمستخدمة والمستخدم والمستخدمة والمستخدمة والمستخدمة والمستخدم والمست
(SEQ ID NO: 113)	KRRRA*SKD	P-NMDARI (Ser896)
(SEQ ID NO: 114)	KHKHS^HKE	PH28 (Sei32)
(SEQ ID NO: 115)	YRY*29992	P.CREB (Sert33)
		him man more and the wife of the live has been
(SEQ ID NO: 116)	GWKNS*IRH	PAFX (Serigo)

FIG. 14

Western blot analysis using a context-independent phospho-PKC consensus substrate motif antibody.



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FIG. 15

Western blot analysis of whole cell lysates using a context-independent antibody specific for the phospho-PKC consensus substrate motif.

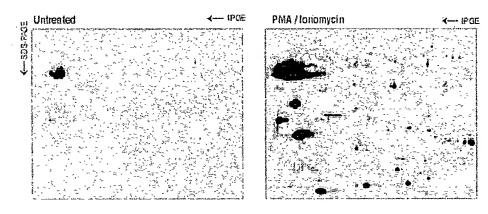
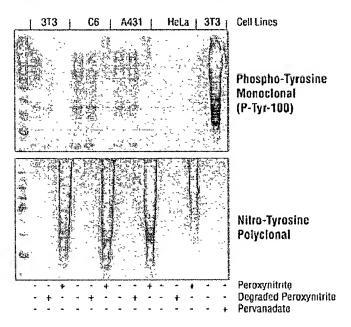


FIG. 16

Western analysis of whole cell lysates using a phosphotyrosine and nitrotyrosine - specific context-independent antibodies.



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FIG. 17

 $Immunocytochemical\ staining\ of\ NIH/3T3\ cells\ using\ a\ polyclonal\ context-independent\ antibody\ specific\ for\ nitrotyrosine\ (brown)\ .$

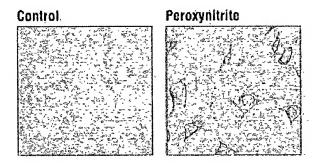


FIG. 18Phosphothreonine-X-arginine motif-specific context-independent antibody ELISAs.

		5 10 15 20 25
(SEQ ID NO:	117)	GLT*VK C.Ral (T481)-P
		LAT*VK 128 (7491) Bassal
(SEQ ID NO:	119)	FFT*RH PKC (Th/639)-P
(SEQ ID NO:	120)	PLT*PR ERF (This26) P
(SEQ ID NO:	121)	NVT*MR Caspase-7 (Thr44)-P
(SEQ ID NO:	122)	AVT*PK APP1 (Thr668).P

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FIG. 19

Western blot analysis or Jurkat cell extracts using a context-independent antibody specific for the phosphothreonine-X-arginine motif.

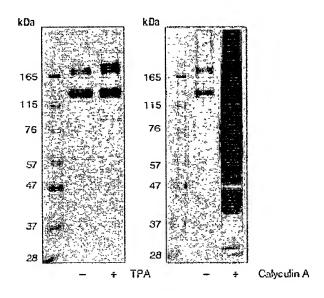
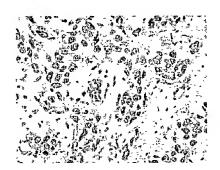


FIG. 20

IHC staining of proteins containing phosphorylated threonine-X-arginine motifs in human breast carcinoma.



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FIG. 21

Western blot analysis of calyculin A treated A431 cells, using a context-independent antibody specific for the phospho-14-3-3 binding motif #2 (phospho(Ser)-Arg-X-(Tyr/Phe)-X-pSer).

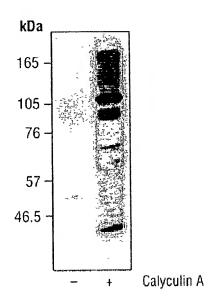


FIG. 22

Phospho-ATM/ATR consensus substrate motif-specific, context-independent antibody ELISAs.

	0 5 10 15 20
(SEQ ID NO: 123) PLS*QE	2
(SEQ ID NO: 124) YPS#QE	BRCA1 (\$1524)
(SEQ ID NO: 125) VST*QE	Chk2 (T68)
(SEQ ID NO: 126) SVT*QS*QG	Chk2 (T26/\$28)
(SEQ ID NO: 127) PIS"QN	Brc (\$1466)(3)
(SEQ ID NO: 128) SFS*QP	5 Chết (S345)
(SEQ ID NO: 129) SSS*QP	Chk1 (\$317)
(SEQ ID NO: 130) DLS*QV	C-Abl (S465)
(SEQ ID NO: 131) SLS*QG	295/NBS1 (S343)

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FIG. 23

Western blotting of COS cell extracts using a context-independent antibody specific for phospho-ATM/ATR consensus substrate motif.

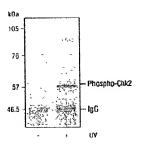


FIG. 24

Western blot analysis of UV treated COS cells, using a context-independent antibody specific for phospho-ATM/ATR consensus substrate motif.

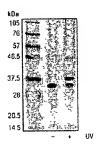


FIG. 25

Phospho-14-3-3 binding motif-specific, context-independent monoclonal antibody ELISAs (T* and S* denote phospho-threonine and serine).

			O	5	10	15	20	25	30	35	5
(SEQ ID NOS:	132)	YRSPS*MP	-			2 2 2	2-2-5			,-·	-cdc25 (S216)
(SEQ ID NOS:				₹- \[- 1 #Z			3 27	Te, <u>"</u>	7	,	Bad (S136)
(SEQ ID NOS:		PRTTS*FA	┪	-cim payer	74.	- 1	128 V	T 4 -7 -7	.~ .	٠ - *	GSK-3β (S9)
(SEQ ID NOS:		SRHST*YP						- :			Bad (S1,12)
(SEQ ID NOS:	136)	QRSTS*TP				tair and		وأراء والمتاس	• [-		Ral-1 (S259)
(SEQ ID NOS:	137)	LRSIS*LP						===:	. M. A	~ ; `	ASK (S967)

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FIG. 26

Western blot analysis of calyculin A treated A431 cells, using a context-independent antibodies specific for phospho-14-3-3 binding motif #1.

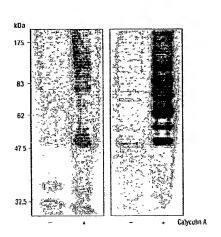


FIG. 27

Phospho-PDK1 docking motif-specific, context-independent monoclonal antobody ELISAs.(I* and S* denote phospho-threonine and serine.)

								PDK	1 Dock	cing M	otif						
		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
(SEQ ID NOs: 138)	FLGFS*Y		A 76	8,540.10					econo vene	oga - sunga di	A. at Popul	Varte de	en sociologic	التوميين	3-7x-1 -	, 3m	⊶ోSĞK;``.
(SEQ ID NOs: 139)			- Processor		~				X.7. 4		-داني-ترد		F. 1 8	ř.	[4, 13]	. 2	· PKC0;
(SEQ ID NOs:140)	FRNFS."Y						· · · · · · · · · · · · · · · · · · ·		3-4	and below-th						à. 'S	≥- ĴPKOĝ ≛
(SEQ ID NOs: 141)	FQGFT*Y	<u> </u>						في شهر پښتورو ده		THE PARTY	-THEN T-P	-			E. S.	? . * - *-	PKCy
(SEQ ID NOs: 142)	FAGES Y		75.67	w.#* v	*											<u>-</u> -3,-3	г ⁴РКСβ∙
(SEQ ID NOs: 143)	FLGFT*Y]==	, ř			-, -	15.27						1-3-1			. ;-	p70 S5 K
(SEQ ID NOs: 144)	FSHFT*F					-		· · · · · · · · · · · · · · · · · · ·	32.5			9. T. Y.				-	eEF-2 K
(SEQ ID NOs: 145)	FPQFS*Y		TĮ.		2.2.5	7	V										- Akt

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FIG. 28

Western blot analysis of extracts from A431 cells using a monoclonal context-independent antibody specific for the phospho-PDK1 docking motif.

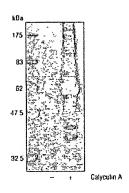


FIG. 29

Immunoprecipitation of extracts from NIH/3T3 cells using a monoclonal context-independent antibody specific for the phospho-PDK1 docking motif.

